Anaerobik Glikoliz HL-60 Akut Promyelositik Lösemi Hücrelerinde Enerjinin Oluşumu için Temel Yoldur

Anaerobic Glycolysis is the Main Pathway for Energy Generation in Hl-60 Acute Promyelocytic Leukemia Cells

HL-60 Akut Lösemi Hücrelerinde Anaerob Glikoliz / Anaerobic Glycolysis in HL-60 Acute Leukemia Cells

Hakan Boyunaga [1], Hatice Keles [2], Levent Kenar[3], Ali Ugur Ural[4], Ferit Avcu[4]

[1]Department of Biochemistry and Clinical Biochemistry, Kırıkkale University Faculty of Medicine, Kırıkkale,

[2]Departments of Internal Medicine, Kırıkkale University Faculty of Medicine, Kırıkkale,

[3]Department of Biochemistry and Medical NBC Defense, Gulhane School of Medicine, Ankara,

[4]Department of Hematology, Gulhane School of Medicine, Ankara, Turkey.

Özet

Amaç

Fizyolojik koşullarda, normal hücreler enerjilerini sağlamak için başlıca glikolitik aerobik yolağı kullanırlar. Ancak, birçok kanser hücresi enerji üretimi için anaerobik glikolitik yolağı kullanır. Bu çalışmada, HL-60 akut promyelositik lösemi hücrelerinin enerji üretiminde karbonhidrat yolağının araştırılması amaçlanmıştır.

Gereç ve Yöntemler

Lösemi hücreleri normal lökositler gibi aerobik ve anaerobik koşullarda radyoaktif işaretlenmiş glukoz ile inkübe edildi ve glikojen tüketimi, CO_2 veya laktata parçalanan radyoaktif işaretli glukoz oranları, yani aerobik veya anaerobik glikoliz oranları saptandı.

Bulgular

Glikojen tüketimi aerobik lösemi hücre kültüründe normal lökosit hücre kültürlerindekine göre anlamlı derecede yüksek bulundu (P<0,01). Aerobik koşullardaki lösemi hücrelerinde anaerobik glikoliz oranı %93,8 di ve glikojenin kullanımı ile anaerobik koşullarda %7,31 oranında arttırılmış glikojenin kullanılmasıyla bu oran %96,6 ya çıkmıştır.

Sonuç

HL-60 premyolositik lösemi hücrelerinde temel olarak anaerobik glikolizin enerji üretimi için daha etkin olduğu söylenebilir. Bu sonuç, premyolositik löseminin tedavisinde yeni tedavi yaklaşımların geliştirilmesi için daha kapsamlı çalışmalarla da desteklenirse önemli olabilir.

Anahtar Kelimeler

Enerji Metabolizması, HL-60 Premyolositik Lösemi Hücresi, Glikojen Yıkımı.

Abstract

Aim

In physiological conditions, normal cells use mainly the glycolytic aerobic pathway to provide energy. However, most cancer cells utilize anaerobic glycolytic way for energy generation. Aim of this study was to investigate the carbohydrate metabolic pathways of HL-60 acute promyelocytic leukemia cells for energy production.

Material and Methods

Leukemia cells as well as normal leukocytes were incubated with radiolabelled glucose in aerobic and anaerobic conditions and glycogen consumptions and the ratios of radiolabelled glucose catabolized into CO_2 or lactate, that is, the rates of aerobic or anaerobic glycolysis, were determined.

Results

The glycogen consumption was significantly higher in aerobic leukemia cell culture than normal leukocyte culture (p<0.01). The rate of anaerobic glycolysis was 93.8% in leukemia cells in aerobic conditions and it increased to 96.6% while utilization of glycogen increased by 7.31% in anaerobic conditions.

Conclusion

In conclusion, principally anaerobic glycolysis is effective for energy generation in HL-60 promyelocytic leukemia cells. This result may be important for the development of new therapeutic approaches in the treatment of promyelocytic leukemia, requiring further comprehensive studies.

Keywords

Energy Metabolism, HL-60 Promyelocytic Leukemia Cell, Glycogen Degradation.

DOI: 10.4328/JCAM.10.2.12 **Received:** 02.09.2009 **Accepted:** 15.10.2009 **Printed:** 01.05.2010 J.Clin.Anal.Med. 2010;1(2): 5-8

Corresponding author: Hakan Boyunaga. Department of Biochemistry and Clinical Biochemistry, Kırıkkale University Faculty of Medicine, Kırıkkale, Turkey. **Phone:** +90 312 304 33 29, **E-mail:** hboyunaga@kku.edu.tr

Introduction

Some structural and metabolic alterations can occur with malign transformation of a normal living cell [1]. Metabolic changes may appear primarily in pathways of energy formation for the cell. Glucose and glutamine are the common sources of energy for both normal and malign cells [2].

Glycolysis and cell respiration function in both ATP synthesis and metabolic pathways of carbohydrates and other compounds. Both normal and cancer cells use similar metabolic pathways for energy production but in varying rates [3]. In 1930, it was firstly reported by the Warburg that anaerobic glycolysis rate was increased in cancer cells [4]. Some recent studies also indicated that cancer cells predominantly use anaerobic glycolysis rather than aerobic one for their energy generation but the rates differ [5-7]. For the increased protein synthesis and cell division, tumor cells function to provide energy by metabolizing the nutrients at an increased rate. To meet the requirements of increased metabolism, it may be beneficial for tumor cells to use the anaerobic glycolytic pathway, a quicker way to generate energy. But, on the other hand, they consume the organism's supplies in a short period of time resulting in cashectia.

The main way through which cells metabolize is mostly dependent on oxygen level in the medium. In the case of high oxygen level, glucose is catabolized via aerobic glycolytic pathway followed by the insertion of degradation products into the Krebs cycle. Then, phosphate with high energy is included into the electron transport chain resulting in energy gain [8,9]. When oxygen concentration is inadequate, the anaerobic glycolytic pathway, which is less efficient than aerobic glycolysis, is completely activated, and cells maintain their viabilities even under low oxygen conditions [8]. Depending on the oxygen level, the selection of either the aerobic or anaerobic pathway in ATP synthesis is called "Pasteur Effect" [10-14]. The possession of this effect by tumor cells may provide them to maintain their viabilities, thus may influence the success of the treatment with anti-neoplastic agents which block energy metabolism of tumor cells. So, this study aimed to investigate the carbohydrate metabolic pathways of HL-60 acute promyelocytic leukemia cells for energy production, thus to provide appropriate selection and development of antineoplastic agents targeting energy metabolism of these cells to increase the effectiveness of the treatment.

Material and Methods

This study was performed within the collaboration of Departments of "Biochemistry and Clinical Biochemistry" and "Internal Medicine" of Kırıkkale University School of Medicine and Departments of "Hematology" and "Biochemistry and Clinical Biochemistry" of Gulhane School of Medicine.

Chemicals and biomaterials

D-[6-C14] glucose was purchased from Amersham Company and hexokinase and glucose-6-phosphate dehydrogenase enzymes were from Boehringer. All other chemicals used were in analytical grade. HL-60 acute promyelocytic leukemia cells were provided from Memorial Sloan-Kettering Cancer Center NY, NY and maintained at 37 °C in the medium of 5% CO_2 and medium of RPMI 1640 supported by fetal calf serum 10%, 2 µM L-Glutamine, 100 µg/mL streptomycine and 100 U/ml penicilline. A total of seven cultures from HL-60 acute promyelocytic leukemia cells were prepared. For the preparation of normal leukocyte cultures blood sample was obtained from a healthy subject and put in tubes with acid-citrate-dextrose (ACD). Then leukocyte isolation was performed by the method described previously by English and Anderson [15] and a total of seven cultures from normal leukocytes were prepared.

Radioactive incubations and analysis of excreted end products

Radioactive incubations were performed by glucose in which sixth carbon was labelled with radioactive Carbon 14 (D-[6-C14] glucose). Amount of labelled glucose added to each culture was 25µCi D-[6-C14] glucose. Before the incubation procedure, both the normal leukocyte cultures and the HL-60 cell cultures were made up to adequate concentrations and each cell culture was separated into three groups. The first one was for the aerobic culture the second one was for the anaerobic culture after KCN addition defined by Tielens [9] and the last one was for the determination of the initial glycogen and protein contents of the cells. Both aerobic and anaerobic cell cultures were immediately incubated with radiolabelled glucose for 4 hours in a specially designed chamber at 37 °C. The cultures which were separated for the determination of zero time glycogen and protein levels were not incubated with radiolabelled glucose. Incubated cell cultures which catabolize externally given radioactive glucose through glycolysis convert it into products including lactate, acetate and propionic acid. Following incubation, the generated radioactive CO_2 was collected in scintillation vials via nitrogen gas. Then, the content was separated as supernatant and pellets. By using supernatant layer, end-products of glycolysis (lactate, acetate and piruvate) were collected in scintillation vials by anion-exchange chromatography, and were calculated on standard graphics in Microsoft Excel program.

In pellets, protein was determined by modified Lowry method and glycogen by Hassid and Abraham's enzymatic method. Glucose in supernatant was measured enzymatically by glucose oxidase method. Glycogen consumption and CO_2 production for each gram of protein were calculated by using data obtained by the measurement of glycogen and protein found in the pellets.

Statistical Analyses

Statistical analysis of the data was done with 9.0 SPSS Package programme for computer. Kruskal-Wallis and Mann Wittney U tests were used for the difference between groups. $P<0.05$ was assumed to be significant.

Results

Table1 shows the amounts of glycogen consumption, labelled and total end products of glycogen metabolism, CO_2 and lactate, and external glucose degradation as percentage of internal glycogen degradation in normal and leukemic cell lines in both aerobic and anaerobic media. Glycogen consumption was significantly higher in anaerobic leukocyte culture than aerobic one ($p<0.01$). Labelled and total amounts of end product lactate were higher ($p<0.01$ and $p<0.01$, respectively) and labelled and total amounts of end product CO_2 were lower ($p<0.01$ and $p<0.01$, respectively) in anaerobic leukocyte culture when compared to aerobic one.

When aerobic HL-60 promyelocytic leukemia cell culture was compared with aerobic normal leukocyte culture, glycogen consumption was found to be significantly higher ($p<0.01$), labelled and total end product lactate was found to be higher ($p<0.01$) and labelled and total end product CO2 was found to be lower in leukemia cell culture ($p<0.01$). When aerobic HL-60 promyelocytic leukemia cell culture was compared with anaerobic normal leukocyte culture, glycogen consumption was still significantly higher ($p<0.01$) but both labelled end products lactate and CO2 were lower ($p<0.01$ and $p<0.01$, respectively) while both total end products lactate and CO2 were higher ($p<0.01$ and $p<0.01$, respectively) in leukemia cell culture.

In anaerobic HL-60 promyelocytic leukemia cell culture glycogen consumption was significantly higher ($p<0.05$) and both labelled and total amounts of end product CO_2 were lower ($p<0.01$ and $p<0.01$, respectively) while both total and labelled amounts of end product lactate were higher ($p<0.01$ and $p<0.01$, respectively) than the ones in aerobic HL-60 promyelocytic leukemia cell culture. The findings were the same for the comparison of anaerobic HL-60 promyelocytic leukemia cell culture with aerobic and anaerobic normal leukocyte cultures.

Table 1. Utilization of glycogen and production of CO_2 and lactate by normal leukocytes and HL-60 promyelocytic leukemia cells in aerobic and anaerobic cultures.

	Glycogen degradation (pmol glu/h/µg prt) mean±SD	Labelled end-products (pmol glu/h/µg prt) mean±SD		Total end-products (pmol glu/h/µg prt) mean±SD		External glucose degradation (% of internal glycogen degradation)
		CO_2	Lactate	CO_2	Lactate	
Aerobic-Leucocyte culture (n=7)	927±17	78.7±4.3	3 .07±0.7	4979±276	94±570	8.83±1.13
Anaerobic-Leucocyte culture (n=7)	1562±68	9.3±2.0	83.6±2.5	334±14	3012±204	5.94±0.79
Aerobic-HL-60 acute promyelocytic leukemia cell culture (n=7)	2660±93	5.3±1.3	81.4±2.9	342±22	5206±196	3.26±0.51
Anaerobic-HL-60 acute promyelocytic leukemia cell culture (n=7)	2870±152	3.7±0.6	104.8±5.4	200±14	5673±261	3.78±0.64

Discussion

In the present study analysis of excreted end-products demonstrated that normal leukocytes use primarily the aerobic glycolysis in aerobic conditions but they shift their energy metabolism predominately to anaerobic glycolysis in anaerobic conditions. The results of this study also indicated that HL-60 promyelocytic leukemia cells use primarily the anaerobic glycolytic pathway which gets more predominant in anaerobic conditions.

Warburg first reported in 1930 that cancer cells possess anaerobic glycolysis in high rate [4]. Later on numerous studies were performed to investigate in details how carcinogenic cells use the metabolic pathways to generate ATP when compared to the function of original stem cells [16]. Indeed, the purpose of these studies was to develop more efficient chemotherapeutic agents. The findings indicated that cancer cells often use different metabolic ways as compared to those used by original cells. But, it was also found that a majority of cancer types use essentially anaerobic glycolysis in different rates, and their cell division frequency is quite high [17,18]. The metabolic rate of some cancer cells may be increased up to approximately 400 times [7]. This increase in metabolism also causes increases in enzyme activities of control points in glycolysis like hexokinase, phosphofructokinase and piruvate kinase [19]. Similar increases may also be seen in glucose carrier molecules [18]. Moreover, as addressed by Warburg [4], high rate of anaerobic glycolysis might not be associated with all types of cancer cells and a rather low cell division rate with a normal glycolysis could be seen in some cancer types like Morris hepatoma [20, 21]. The rates of metabolic pathways the cells use can be different although they function on some common ways in principal.

Oxygen level and nutrient concentration are normally the main biological indicators which determine the exact metabolic pathway the cell can use to obtain ATP [8]. The main advantage of the use of anaerobic glycolysis with respect to tumor cell is to obtain ATP easier but it is an ineffective pathway. While 36-38 moles of ATP per mole of glucose are obtained through aerobic glycolysis only 2 moles of ATP per mole of glucose are obtained through anaerobic glycolysis. Therefore 19 times more substrate is utilized in anaerobic glycolysis to obtain the same amount of ATP obtained through aerobic glycolysis.

The conversion of the metabolism of normal or cancer cells from aerobic glycolysis into anaerobic glycolysis in reduced level of ambient oxygen, the so called Pasteur Effect, can accelerate the consumption of glycogen and lipid stores [22, 23]. Moreover, as a result of this

alteration, the effect of chemotherapy used to regulate the energy metabolism of cancer cells remains limited [24-26].

In our study, as it was reflected by the level of radiolabelled end product CO_2 aerobic glycolysis was the predominant way of metabolism (96%) in aerobic leukocyte culture. But in anaerobic media leukocytes shifted their energy to mainly anaerobic glycolysis (90%) with an increase in consumption of glycogen by 73.8%, showing prominent Pasteur Effect. The ratios of radiolabelled glucose catabolized into CO_2 or lactate, that is, the rates it was directed to either anaerobic or aerobic way were also investigated on aerobic HL-60 leukemia cell line and significant differences were found when compared to anaerobic and aerobic control leukocyte cultures. The rate of aerobic glycolysis was only 6.2% in aerobic conditions that is the main way of glycolysis was anaerobic (93.8%) in HL-60 promyelocytic leukemia cells. The level of anaerobic glycolysis increased to 96.6%, and utilization of glycogen increased by 7.31%, when oxygen use of HL-60 promyelocytic leukemia cells was inhibited by KCN.

These results indicate that HL-60 acute promyelocytic leukemia cells metabolize glucose prominently through the anaerobic glycolysis and endogenous glycogen degradation is the main pathway for energy generation. These results also indicate that the Pasteur Effect is also validated in HL-60 promyelocytic leukemia cells.

In conclusion, it is evident that principally anaerobic glycolysis is effective for energy generation in HL-60 promyelocytic leukemia cells in aerobic conditions. On the other hand, the rate of anaerobic glycolysis gets more prominent in anaerobic conditions. This result may be important for the development of new therapeutic approaches in the treatment of acute promyelocytic leukemia, requiring further comprehensive studies.

References

1. Fischer CP, Bode BP, Souba WW., Adaptive Alterations in Cellular Metabolism with Malignant Transformation. Annals of Surgery, 1998, 227, 627-63.
2. Sauer L, Stayman J, Dauchy R. Amino acid, glucose, and lactic acid utilization in vivo by rat tumors. Cancer Res, 1986 , 46, 3469-3475.
3. Dienel GA, Hertz L. Glucose and lactate metabolism during brain activation. J Neurosci Res, 2001, 66, 824-838.
4. Warburg O, Metabolism of Tumors. London: Arnold Constable., 1930
5. Rossignol R, Gilkerson R, Aggeler R, Yamagata K, Remington SJ, Capaldi RA. Energy substrate modulates mitochondrial structure and oxidative capacity in cancer cells. Cancer Res, 2004, 64, 985-993.
6. Raghunand N, Gatenby RA, Gillies RJ. Microenvironmental and cellular consequences of altered blood flow in tumours. Br J Radiol, 2003, 76, 11-22.
7. Newsholme EA, Board M, Application of metabolic-control logic to fuel utilization and its significance in tumor cells, Adv Enzyme Regul, 1991,31, 225-246.
8. Tielens AGM. Energy generation in parasitic helminths. Parasitology Today, 1994,10, 346-352.
9. Tielens AGM, Pas FAM, Heuvel JM, van den Bergh SG. The aerobic energy metabolism of Schistosoma mansoni miracidia. Molecular and Biochemical Parasitology, 1991,46, 181-184, .
10. Hardie DG., Metabolic control: a new solution to an old problem. Curr Biol, 2000,19, 757-759.
11. Seagroves TN, Ryan HE, Lu H, Wouters BG, Knapp M, Thibault P, Laderoute K, Johnson RS. Transcription factor HIF-1 is a necessary mediator of the pasteur effect in mammalian cells. Mol Cell Biol, 2001,21, 3436-3444, .
12. Herting B, Meixensberger J, Roggendorf W, Reichmann H., Metabolic patterns in meningiomas, J Neurooncol, 2003,65, 119-123.
13. Summers JE, Ratcliffe RG, Jackson MB. Anoxia tolerance in the aquatic monocot Potamogeton pectinatus absence of oxygen stimulates elongation in association with an unusually large pasteur effect. J Exp Bot, 2000,51, 1413-1422, .
14. Boyunaga H, Schmitz MGJ, Brouwers JFHM, Van Hellemond JJ, Tielens AG. Fasciola, Hepatica miracidia are dependent on respiration and endogenous glycogen degradation for their energy generation. Parasitology, 2001,122, 169-173.
15. English D, Anderson BR., Single step separation of red blood cells, granulcytes and mononuclear leukocytes on discontinuous density gradients of Ficoll-Hypaque. J. Immunol. Methods, 1974,5, 249-259.
16. Chesney J, Mitchell R, Benigni F, Bacher M, Spiegel L, Al-Abed Y, Han JH, Metz C, Bucala R., An inducible gene product for 6-phosphofructo-2-kinase with an AU-rich instability element: role in tumor cell glycolysis and the Warburg effect. Proc Natl Acad Sci USA, 1999, 96, 3047-3052.
17. Dang CV, Lewis BC, Dolde C, Dang G, Shim H., Oncogenes in tumor metabolism, tumorigenesis, and apoptosis. J Bioenerg Biomembr, 1997, 29, 345-354.
18. Eigenbrodt E, Reinacher M, Scheefers-Borchel U, Scheefers H, Friis R., Double role for pyruvate kinase type M2 in the expansion of phosphometabolite pools found in tumor cells. Crit Rev Oncog, 1992,3, 91-115.
19. Kress S, Stein A, Maurer P, Weber B, Reichert J, Buchmann A, Huppert P, Schwarz M. Expression of hypoxia-inducible genes in tumor cells. J Cancer Res Clin Oncol, 1998,124, 315-320.
20. Parry DM, Pedersen PL. Intracellular localization and properties of particulate hexokinase in the Novikoff ascites tumor. Evidence for an outer mitochondrial membrane location. J Biol Chem, 1983,258, 10904-10912.
21. Morris HP. Chemically induced hepatoma cell line. Recent Results in Cancer Research, 1954,44, 103-114, .
22. Hochachka PW., Mechanism and evolution of hypoxia-tolerance in humans. Journal of Experimental Biology, 1998,201, 1243-1254.
23. Scatena R, Bottoni P, Martorana GE, Ferrari F, De Sole P, Rossi C, Giardina B. Mitochondrial respiratory chain dysfunction, a non-receptor-mediated effect of synthetic PPAR-ligands: biochemical and pharmacological implications. Biochem Biophys Res Commun, 2004,319, 967-973.
24. Iwata K, Ogata S, Okumura K, Taguchi H. Induction of differentiation in human promyelocytic leukemia HL-60 cell line by niacin-related compounds. Biosci Biotechnol Biochem, 2003,67, 1132-1135, .
25. Morshed SR, Tokunaga T, Otsuki S, Takayama F, Satoh T, Hashimoto K, Yasui T, Okamura M, Shimada J, Kashimata M, Sakagami H. Effect of antitumor agents on cytotoxicity induction by sodium fluoride. Anticancer Res, 2003,23, 4729-4736.
26. Scatena R, Bottoni P, Vincenzoni F, Messana I, Martorana GE, Nocca G, De Sole P, Maggiano N, Castagnola M, Giardina B. Bezafibrate induces a mitochondrial derangement in human cell lines: a PPAR-independent mechanism for a peroxisome proliferator. Chem Res Toxicol, 2003, 16, 1440-1447.